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Mice in which the gene for oxytocin has been deleted (OT -/- mice) have been used to study mammary gland development in the absence of this hormone. These mice, derived from 129 57/B16 background, have no processed OT and thus the mammary gland is not exposed to OT at any stage of development. The animals are being studied to determine if any morphological changes occur throughout the life cycle of mouse mammary gland development in the absence of this hormone. In addition, we are also testing whether deficiency of OT and the absence of its effects upon the mammary gland, may in part, predispose the breast to neoplastic changes. To date we have sacrificed both virgin and parous (OT -/-) and age-matched wild types cohorts (OT +/+) throughout the life span of the animals (3 mo to 24 mo). Histological analysis of these mammary tissues is currently in progress.

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Title: Mammary Gland Ontogeny and Neoplasia in Oxytocin Defoicient Mice

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INTRODUCTION

Oxytocin (OT), a nine amino acid peptide, is synthesized within the paraventricular and supraoptic nuclei (PVN and SON) of the hypothalamus and stored within the posterior pituitary gland ( ). Suckling by the young during lactation is the primary stimulus for the release of OT. Once released, OT attaches to its receptor on the mammary myoepithelial cell and elicits contraction and the ejection of milk ( ). In addition to its contractile effects upon myoepithelial cells, OT has been reported to enhance myoepithelial cell differentiation and proliferation in murine mammary organotypic cultures ( ). These effects of OT were most apparent in animals that were primed with estrogen and progesterone ( ).

The pattern of mammary gland differentiation and proliferation in the presence and absence of OT has not been tested *in vivo*. Mice in which the gene for OT has been deleted (OT -/-) provide a novel and direct means of assessing the effects of OT deficiency upon the mammary gland. OT null mice are fertile and deliver their progeny

normally at term, but are unable to nurse their young ( ). Although milk production occurs, milk ejection does not ( ). The progeny of OT -/- mice will die of nutritional deprivation unless placed with a surrogate lactating female that is able to release OT and eject milk. Careful study of the mammary histology and differentiation at sequential stages in the life span of the OT -/- mouse compared to its wild type cohort (OT +/+) has not yet been reported. In the present study we investigated the ontogeny of mammary development in both parous and virgin OT -/- mice and also determined whether deficiency of OT and the absence of its effects upon the mammary gland may, in part, predispose the breast to neoplastic changes.

PROGRESS TO DATE

Female OT (-/-) and OT (-/-) mice of C 57BL/6 background were used for these studies ( ). The OT -/- mice were generated by Dr Scott Young, NIMH ( ), and breeding pairs were purchased from Jackson Laboratories (Bar Harbor,ME). Animals were bred and housed for this study in the viral free quarters of the University of Pittsburgh Animal Facility under a 12-h light, 12-h dark cycle (lights on at 0700 h). Mice were housed in standard suspended or shoebox cages in groups of up to five animals per cage with free access to food (standard rodent chow) and water. The studies were

approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Both virgin and parous (one prior pregnancy) OT -/-, OT +/+, and OT +/- mice were sacrificed in groups of 5-6 at 6, 12, 18, 24 months of life, whereas only virgin mice were sacrificed at 3 months of life. For mating, up to three 2-month old females were placed in a cage with one male. After 16 days, mice were checked daily for pregnancy. When parturition seemed imminent, each female was removed to an individual cage for delivery. Since OT-/- are unable to nurse young, they were returned to group housing as soon as delivery had been observed and were maintained until they reached the designated age for sacrifice in this study. OT +/+ and +/- (heterozyge) mice nursed their pups for approximately 24-26 days. After weaning, dams were again housed in groups of up to five per cage until they had reached the appropriate age for sacrifice in this study.

Animals were killed rapidly by scissors decapitation. After killing, a single cut was made in the skin from the tail to the neck, and the skin retracted and pinned in place to reveal the breast tissue. The right anterior breast was lifted and a small cut was made in the posterior end of the connective tissue. Blunt scissors dissection was used to loosen the breast tissue from the skin. When completely separated, sharp scissors dissection was used to free the breast tissue from surrounding connective tissue. The left anterior mammary glands were used from each animal.

Breast tissue was placed into a vial containing 4% formalin in phosphate buffered saline and allowed to soak overnight. Formalin-soaked tissue was placed into a cassette, sequentially dehydrated for 30 min each in 70%, 80%, 95%, 100% solutions of ethanol, and taken through two changes of Hemo-De (Fisher Scientific, Pittsburgh, PA) prior to immersion in melted embedding media (Tissue Path Paraplast X-tra, Fisher) for one hour at 57°C. Following this step, tissue was placed into an embedding mold that had been treated with Histo Prep mold releasing agent (Fisher Scientific). A labeled tissue cassette was placed on top of the mold and the unit was filled with embedding media, which was allowed to solidify overnight. The tissue cassette was removed from the mold the following day. Tissues were cut and subsequently stained with hematoxylin-eosin. Immunoperoxidase staining with antibodies to smooth muscle actin (specific for myoepithelial cells) and keratin (specific for epithelial cells) were used to identify cell types. Blood vessels were identified by an antibody to Factor VIII.

Histological analysis of mammary tissue was performed on serial sections under the light microscope by two independent investigators. The analysis focused upon ductal structures, lobule development and periductal capillaries. The rating scale was as follows: Lobule development: 1 = none; 2= rare (TDLU); 3= 1-2 TDLU; Periductal capillaries: 1= up to 1/ duct or lobule; up to 2-3/ duct or lobule; >4 / duct or lobule. Based upon the rating scale an overall rank score was assigned to each tissue sample.

Samples were analyzed by multivariate ANOVA using age, genotype and parity as variables. When ANOVA indicated significant differences among groups, pairwise comparisons between groups were made by *post hoc* Fisher's protected least significant differences (PLSD) test.

At the time of writing of the proposal the analysis is in progress. Our time table is to conclude the analysis by July 2001.